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13. ABSTRACT (Maximum 200 Words)
Although considerable progress in the understanding of prostate cancer has been made in the last few years, the basic knowledge of the biology of this disease remains elusive. The development of this cancer is related to the male sexual hormone (testosterone) but the actual mechanisms by which testosterone affects the development of this cancer is not known.

The prostate gland has at least three different types of cells that contribute to the physiology of the gland: basal, luminal, and neuroendocrine cells. It is not totally clear what the relationship is between these different cell types, how testosterone affects them and which one is the target cell in prostate cancer development.

We will use new transgenic technology that allows tagging of a particular cell population and following its behavior over the life of the animal. These experiments will be performed in mice because this technology is well developed in these animals and there is a basic knowledge of the rodent prostate.

The studies proposed here will clarify some of the basic aspects of the biology of the prostate gland and the process of carcinogenesis in this organ.

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INTRODUCTION

The purposes of this research are to investigate cell lineages in the prostate gland of the mouse and to identify the cellular origin of prostate adenocarcinomas. The prostate epithelium contains, among others, luminal and basal cells. It is believed, but not proven, that a subpopulation of the basal cells may be stem cells (*1*) and that basal cells differentiate into luminal cells (*2, 3*). On the other hand, prostate cancer cells have characteristics of luminal cells (*4*). This project aims to use Cre/loxP technology (Sauer, 1998) to develop mice in which different prostate cell subpopulations are permanently labeled. Hereto, mice have been generated that express, constitutively or upon tamoxifen treatment, Cre recombinase under the control of keratin 5 (K5) or K14 promoters (active in basal cells (*6*)) or the probasin (PB) promoter (active in luminal cells of adult male mice (*6*). These mice have been crossed with ROSA26 mice (*7*), which harbor a universal promoter driving a β-galactosidase (β-gal) reporter gene preceded by a floxed stop sequence. In the resulting litter, Cre-mediated recombination is expected to remove the stop sequence and lead to permanent labeling of a cell subpopulation with β-gal.

BODY

Statement of Work – Specific Aim 1

The mice described in Specific Aim 1 of the approved Statement of Work (SOW), bigenic (K5-CreER^{T2} x ROSA26) mice and bigenic (PB-Cre x ROSA26) mice, were generated and tested. As a backup for the bigenic (K5-CreER^{T2} x ROSA26) mice, we also generated and tested bigenic (K14-CreER^{T2} x ROSA26) mice.

<u>Generating and Testing Bigenic (K5-CreER^{T2} x ROSA26) and Bigenic (K14-CreER^{T2} x ROSA26) Mice</u>

Three lines of the parental strain K5-CreER^{T2} were developed using reagents from Dr. P. Chambon's laboratory. Dr. Chambon also provided the parental strain K14-CreER^{T2} (*8*). The third parental strain, ROSA26 (*7*), was obtained from the M. D. Anderson Animal Facility in Houston with permission from Dr. P. Soriano. The parental strains K5-CreER^{T2} and K14-CreER^{T2} were each crossed with ROSA26. Tail DNA was isolated from the progeny and tested for the presence of Cre and β-gal by PCR analysis.

Five bigenic (K14-CreER^{T2} x ROSA26) mice and two wild type control animals were treated with 4-hydroxytamoxifen (4-OHT; 1 mg in 200 μl corn oil) intraperitoneally (i.p.) daily for 5 days and were sacrificed 48 hours after the last treatment. We collected dorsal skin (fixed in formalin, frozen in Tissue-Tek® OCT™ Compound (Miles Scientific, Naperville, IL), and snap frozen), heads (fixed in formalin), thymus (formalin and snap frozen), and genitourinary tract (fixed in formalin). Immunohistochemical analysis of β-gal was performed with an anti-β-gal polyclonal antibody (AB1211; 1:1000 dilution; Chemicon, Temecula, CA) in formalin-fixed tissues. We found no staining in the prostate luminal cells and inconclusive staining in the prostate basal cells. The β-gal in the bigenic mice has no nuclear location signal and thus resides in the cytoplasm. The inconclusive staining for β-gal in the prostate basal cells may have been due to the little amount of cytoplasm in these cells.

Five mice of each of the three lines of bigenic (K5-CreER^{T2} x ROSA26) mice were treated with 4-OHT (1 mg in 200 μl corn oil i.p. daily for 5 days) and sacrificed 48 hours after the last treatment. Dorsal skin (fixed in formalin, frozen in Tissue-Tek® OCT™ Compound, and snap frozen), heads (fixed in formalin), thymus (formalin and snap frozen), and genitourinary tract (fixed in formalin) were collected and analyzed for the presence of β-gal by immunohistochemistry. We detected β-gal in the skin but saw marginal reaction in the prostate. Therefore, mice of the bigenic (K5-CreER^{T2} x ROSA26) line with the highest Cre expression in the skin were treated with 4-(1 mg in 200 μl corn oil i.p. daily for 5 days) and sacrificed 48 hours after the last treatment. We did not detect β gal in the prostates of these animals.

Next, we looked into two possible causes for the absence of β gal in the prostate basal cells of the bigenic (K5-CreER^{T2} x ROSA26) mice: lack of recombination (the K5 promoter may not be strong enough in the prostate to induce a critical level of Cre), or low sensitivity of the β gal detection method.

The possibility of lack of recombination was tested by immunohistochemical analysis of formalin-fixed skin, thymus, and urogenital tissues with a polyclonal anti-Cre antibody (69050-3; 1:3000; Novagen, Madison, WI). K5-Cre mice, which constitutively express Cre, served as a positive control. Nuclei in the skin but not the bladder of K5-Cre mice reacted positively with the anti-Cre antibody. No reaction with the anti-Cre antibody was observed in the nuclei of skin, thymus, and bladder collected from K5-CreER^{T2} mice that received 4-OHT i.p. (1 mg in 200 μl corn oil daily for 5 days). In contrast, nuclei in the skin of K5-CreER^{T2} mice that received a daily topical treatment of 1 mg 4-OHT for 5 days were positive for Cre.

We used immunofluorescence as a possibly more sensitive method to detect binding of an anti-β-gal antibody (AB1211; 1:1000 dilution; Chemicon) to formalin-fixed prostate tissue. This technique did not work: the positive controls were negative. In light of the immunohistochemical results with the anti-Cre antibody, we concluded that the Cre levels induced by the K5 promoter in the prostate basal cells are probably too low to produce recombination of the β-gal gene.

Generating and Testing Bigenic (PB-Cre x ROSA26) Mice

The parental strain PB-Cre, which was originally developed at the University of Southern California (*9*), was established as a colony and crossed with ROSA26 mice. Tail DNA was isolated from the progeny and tested for the presence of Cre and β-gal by PCR analysis.

Urogenital tract was collected from four bigenic (PB-Cre x ROSA26) mice and two wild type animals were subjected to immunohistochemical analysis with an anti-β-gal antibody (AB1211; 1:1000 dilution; Chemicon). We obtained a clear, positive signal in the prostate luminal cells in the four bigenic mice and, as expected, no signal in the wild type animals. We did not detect β-gal in the prostate basal cells.

Statement of Work – Specific Aim 2

Work related to Specific Aim 2 has not been initiated yet.

Statement of Work – Specific Aim 3

Work related to Specific Aim 3 has not been initiated yet.

KEY RESEARCH ACCOMPLISHMENTS

- We performed all mouse crosses needed for Specific Aim 1 of the approved SOW and collected tissue samples for β-gal analysis.
- In K5-Cre mice, expression of Cre can be detected immunohistochemically only in basal cells of the skin, not in prostate basal cells.
- In K5-CreER^{T2} mice, Cre expression is induced by topical but not intraperitoneal administration of tamoxifen.
- In bigenic (PB-Cre x ROSA26) mice, β-gal is detected by immunohistochemistry in prostate luminal cells, not prostate basal cells.

REPORTABLE OUTCOMES

We developed the following animal models:

- K5-Cre mice – they carry Cre recombinase under control of the K5 promoter
- ROSA26 mice (homozygous) – they have been developed from Dr. Soriano's—heterozygous—ROSA26 mice and carry, under control of an universal promoter, a β-gal reporter gene preceded by a floxed stop sequence
- K5-CreER^{T2} mice – they carry a construct of the Cre recombinase and a mutated estrogen receptor under control of the K5 promoter
- K14-CreER^{T2} mice– they carry a construct of the Cre recombinase and a mutated estrogen receptor under control of the K14 promoter
- Bigenic (K5-CreER^{T2} x ROSA26) mice – they carry a construct of the Cre recombinase and a mutated estrogen receptor under control of the K5 promoter and a β-gal reporter gene under control of an universal promoter
- Bigenic (K14-CreER^{T2} x ROSA26) mice – they carry a construct of the Cre recombinase and a mutated estrogen receptor under control of the K14 promoter and, under control of an universal promoter, a β-gal reporter gene preceded by a floxed stop sequence
- Bigenic (PB-Cre x ROSA26) mice they harbor Cre recombinase under control of the PB promoter and, under control of an universal promoter, a β-gal reporter gene preceded by a floxed stop sequence

CONCLUSIONS

We generated and tested the lines needed for Specific Aim 1 of the approved SOW, bigenic (K5-CreER^{T2} x ROSA26) mice and bigenic (PB-Cre x ROSA26) mice. As a backup, we also tested bigenic (K14-CreER^{T2} x ROSA26) mice. Immunohistochemical analysis did not detect β-gal in the prostate basal cells of the K5 and K14 bigenic lines. Immunohistochemical analysis of Cre in K5-Cre mice showed that Cre was detectable in the skin but not the bladder of K5-Cre mice. Together, these results suggest that the K5 promoter in prostate basal cells is too weak to generate a critical level of Cre, resulting in a lack of recombination of the β-gal gene. Further, in bigenic (K5-CreER^{T2} x ROSA26) mice, we detected Cre immunohistochemically after topical but not intraperitoneal administration of 4-OHT. In bigenic (PB-Cre x ROSA26) mice, we detected β-gal by immunohistochemistry in prostate luminal cells, not prostate basal cells. In conclusion, the results indicate that the K5 promoter is not suitable for directing gene recombination and expression to the prostate basal cells. In contrast, bigenic (PB-Cre x ROSA26) mice allow permanent labeling of luminal prostate cells and appear an appropriate model to study the fate of these cells during prostate development.

As pointed out by a previous reviewer, the original Statement of Work (SOW) cannot be completed. Because less than one year is left of the grant period, the PI revised the SOW (submitted separately). In short, the revised SOW proposes to cross the bigenic (PB-Cre x ROSA26) mice with the prostate cancer-prone TRAMP mice (*10*) to investigate the role of luminal cells in prostate carcinogenesis.

The knowledge obtained by the PI is of value to the scientific community: his findings that i.p. tamoxifen does not induce Cre expression in bigenic (K5-CreER^{T2} x ROSA26) mice and that the K5 promoter is unsuitable for directing gene expression and recombination to the prostate basal cells were unexpected based on the available literature; furthermore, the bigenic (PB-Cre x ROSA26) mice are a valuable model to study the fate of luminal cells during development of the prostate.

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